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The Histopathological Spectrum of 3592 Corneal Specimen in China

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Abstract

Importance: This is the largest retrospective report of the prevalence of corneal disease by histopathology analysis, which represents a patient population of over 100 million people in China.

Objective: To analyze the prevalence of corneal diseases in China by histopathological examination.

Design: Case series, a retrospective study was performed by histopathological analysis on 3592 corneal specimens from 3589 patients who underwent corneal surgery between January 2001 and December 2012 in Henan Eye Institute. The clinical data and pathological diagnoses were collected.

Participants: 3592 corneal specimens from 3589 patients.

Main Outcome Measure: histopathological analysis.

Results: Infectious keratitis (2506 specimen, 69.77%) was the most common corneal disease by pathologic diagnosis. Of these, 1330 (53.07%) were diagnosed as fungal keratitis, 279 (11.13%) as bacterial keratitis, 258 (10.30%) as viral keratitis, and 26 (1.04%) as acanthamoeba keratitis. The organism of the infection in 567 (22.63%) specimens could not be determined. Specimens diagnosed with non-infectious corneal disease included 318 (8.85%) corneal tumors. Other corneal disease was the diagnosis for the remaining 768 cases (21.38%).

Conclusion: Infectious keratitis, especially of fungal origin, is the most common Infectious corneal disease in China. It is also the major cause for surgical intervention in the treatment of corneal disease.

Keywords: Corneal disease, Histopathology, Infection, Fungal

Introduction

Corneal infection lead to scaring and vascularization are the one of major contributors to vision loss. Previously published data show that the epidemiology of corneal disease varies from country to country and even from one geographic area to another within countries [1-4]. In Canada, Godeiro et al [5] found only one case (0.2%) of fungal infection in 500 corneal button specimens by histolopathologic examination. Of note, 243 (48.6%) of the 500 cases were diagnosed as corneal degeneration, dystrophy or metabolic diseases. Furthermore, in other developed areas, corneal transplantation is largely performed for optical correction [6]. In contrast, fungal and bacterial infections, even trachoma, may be responsible for the majority of cases of corneal related blindness in developing countries. A report from India shows that 44% of corneal ulcers are due to fungal infection, but the incidence of fungal corneal infection in Nepal [7] and Dallas in United States is less than 17% and 9%, respectively, as demonstrated by corneal scraping culture [8]. These differences may reflect the varying areas and diagnostic methods used.

Examination by histopathology is a common method to confirm the diagnosis of infected pathogens; however, there are limited studies of the diagnosis of corneal disease by histopathlogical analysis. The report from Goderiro et al [5] shows the result of an epidemiologic study of 500 corneal specimens by histological diagnosis in North America. In the current study, we sought to evaluate the epidemiologic features in a large number of corneal disease cases in china using histopathological analysis. A total of 3592 corneal specimens were included in the study. All 3589 patients were seen between January 2001 and December 2012 at Henan Eye Institute, and the pathological diagnoses were analyzed. As best of our knowledge, this is the largest retrospective report about corneal disease by histopathology analysis.

Materials and Methods

Patients

The Institutional Review Board (IRB) of the Henan Eye Institute approved our use of human corneal specimens. All procedures conformed to the Declaration of Helsinki for research involving human subjects.

The 3592 (3592 eyes) corneal specimens were collected for retrospective analysis after corneal surgery. The corneal surgery was performed as following: Penetration keratoplasty (1540 eyes, 42.87%), anterior lamellar keratoplasty (1636 eyes, 45.54%), Lamellar resection (109 eyes, 3.04%); other corneal specimens were obtained from evisceration surgery (303 eyes, 8.45%) and enucleation (4 eyes, 0.10%). All surgeries took place between January 2001 and December

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2012 at Henan Eye Institute. The patients included 2356 (65.59%) males and 1236 (34.41%) females; the age of patients ranged from 3 months to 90 years (average: 43.13 years).

Tissue sample processing

Corneal specimens were fixed in 10% buffered neutral formaldehyde and embedded in paraffin. Paraffin sections (3 uM) were stained with Hematoxylin and Eosin (HE). Periodic Acid-Schiff (PAS) staining was performed for the identification of hyphae and spores. Giemsa staining was used for identified bacterial infection.

Histopathological examination

All of the stained corneal sections were reviewed by two ocular pathologists under light microscopy. Histologic changes in all layers of the cornea were observed. The presence or absences of inflammatory cells were recorded. The determination of fungal infection was confirmed by the existence of hyphae and spores. Bacterial infection was confirmed by the existence of bacterium under oil immersion microscope and positive Giemsa staining. Acanthamoeba keratitis was confirmed by the existence of acanthamoeba cyst or trophozoite. Diagnosis of viral infection was based on existing of a mixed infiltrate composed of chronic inflammatory cells, including lymphocytes, neutrophils, and mononuclear phagocytes [9].

Statistics analysis

The Chi-Square test was used to compare the differences in categorical variables among the groups. *P* value less than 0.05 was accepted as significance.

Results

Histopathologic review of 3592 corneal specimens from 3589 patients showed that 2506 (69.77%) specimens were diagnosed as infectious keratitis (Table 1); the incidence of infectious keratitis were significantly high compared with rest of different corneal diseases ($p<0.01$). The second common corneal disease was corneal tumor 318 eyes (8.85%), followed by other 15 corneal diseases 768 eyes (21.38%). Among the other corneal diseases the top three of them were Corneal leukoma 178 eyes (23.18%), Keratoconus 108 eyes (14.06%) and Marginal keratitis 72 eyes (9.38%).

Among the cases of infectious corneal keratitis, fungal keratitis (1330 eyes, 53.07%) was the most common finding by histopathologic examination compared to other category of corneal disease included in the study ($p<0.05$) (Table 2), followed by bacterial keratitis (279 eyes, 11.13%), viral keratitis (258 eyes, 10.30%), Fungal complicated with bacterial keratitis (46 eyes, 1.84%) and acanthamoeba (26 eyes, 1.04%). The major histopathological founding of fungal infection in our specimen was necrotizing keratitis that in general was associated with both an acute and granulomatous inflammatory infiltrate. PAS staining was positive in those cases for revealing the exits of the fungal elements of hyphal structures and spores. In term of bacterial infection, beside the positive response of Giemsa staining, infiltration of polymorphonuclear leukocytes was demonstrated to be the major response of the cornea to bacterial infection in the cases analyzed in the current study in which neutrophils were present in the epithelial layer and stroma of infected cornea. In herpetic keratitis our result showed that it was composed of a variety of inflammatory cells, including lymphocytes, neutrophils and mononuclear phagocytes. Besides inflammatory infiltration neovascularization and fibrosis were seen in some of advanced corneal infection cases.

Table 1: The incidence of corneal disease by pathological analysis.

Pathological diagnosis	n	%
Infectious keratitis	2506	69.77
Corneal tumor	318	8.85
Other corneal diseases	768	21.38
Total	3592	100

Table 2: The pathological classification of infectious keratitis.

Pathological diagnosis	n	%
Fungal keratitis	1330	53.07
Bacterial keratitis	279	11.13
Viral keratitis	258	10.3
Acanthamoeba keratitis	26	1.04
Fungal complicated with bacterial keratitis	46	1.84
Agnogenic infectious keratitis	567	22.63
Total	2506	69.77

Among the subgroup of cases with infectious keratitis, there were 567 eyes (22.63%) whose infections could not be identified by histopathologic analysis. In those cases, no hyphae, spores, bacterium, acanthamoeba cysts, or trophozoite could be found under the light microscope, nor could they be diagnosed as viral keratitis. Although the pathogens could not be defined by the histopathologic method used in those cases, the patients' charts showed that they were treated according to a clinical diagnosis of fungal keratitis (219 eyes), acanthamoeba keratitis (6 eyes), bacterial keratitis (30 eyes) and infectious keratitis with the combination of fungal and bacterial (312 eyes).

Corneal tumor took the second place of corneal disease in our retrospective study. The most common tumor was benign neoplasm (247 eyes, 77.67%). The incidence of corneal dermoid cyst was much higher than that of other types ($p<0.05$), contributing to 75.71% (187 eyes) of the total number of benign neoplasms. Eighteen eyes (7.29%) were diagnosed with epithelial cell proliferation, 18 eyes (7.29%) with pigmented nevus, 14 eyes (5.67%) with squamous papilloma and 4 eyes (1.62%) with proliferation of granulation tissue, 3 eyes (1.21%) with implanted epithelial cyst.

In addition, 71 (22.33%) of the 318 eyes diagnosed with tumor were found to be malignant neoplasms by histopathologic examination. Among these malignant neoplasms, 31 eyes (43.66%) were diagnosed as carcinoma in situ, followed by 21 eyes (29.58%) with squamous cell carcinoma, 17 eyes (23.94%) with atypical hyperplasia and 2 eyes (2.82%) with melanoma. Other tumors such as vascular, neural, myogenic, lipomatous, lymphoid were not seen in the study.

The top ten of corneal diseases included corneal leukoma (178 eyes, 23.18%), keratoconus (108 eyes, 14.06%). Marginal keratitis (72 eyes, 9.38%), corneal dystrophy (63 eyes, 8.2%), Corneal degeneration 56 eyes, 7.29%), Chemical burn (56 eyes, 7.29%), Corneal edema after keratoplasty (53 eyes, 6.90%), Mooren's ulcer (52 eyes, 6.77%), Bullous keratopathy (43 eyes, 4.43%), and Thermal burn (31 eyes, 4.04%). The remaining cases were diagnosed as other, less common corneal diseases (Table 3).

Table 3: The pathological classification of other corneal diseases.

Pathological diagnosis	n	%
Corneal leukoma	178	23.18
Keratoconus	108	14.06
Mooren's ulcer	52	6.77
Marginal keratitis	72	9.38
Chemical burn	56	7.29
Corneal dystrophy	63	8.2
Thermal burn	31	4.04
Corneal degeneration	56	7.29
Bullous keratopathy	34	4.43
Corneal edema after keratoplasty	53	6.9
Corneal staphyloma	22	2.86
Corneal penetrating wound	15	1.95
Corneal vascularization	17	2.21
Corneal foreign body	7	0.91
Corneal melt	4	0.52
Total	768	100

Discussion

This study serves an important purpose as an initial attempt to use histopathologic analysis to profile corneal disease in central areas of China. This is an effort to represent the large patient population in Henan, a province of over 100 million populations. The epidemiological tendencies of corneal disease varies from one country to another [1-3,5,7,8]. Therefore, information of epidemiology input from comprehensive research is important for establishing appropriate diagnostic and therapeutic strategies. In this article, we analyzed 3592 corneal specimens from 3589 patients who underwent corneal surgery at our eye institute between January 2001 and December 2012 with pathological diagnosis. We found the most common corneal disease was infectious keratitis. The majority of corneal infection is caused by fungus, bacterium, virus, and then protozoon in current study. The main contributor to infectious keratitis was fungal keratitis, followed by bacterial keratitis, viral keratitis and acanthamoeba keratitis. As others have noted, the significantly increased incidence of fungal keratitis may be due to the abuse of antibiotics and corticosteroids and to ocular trauma with vegetative material [2-4,8]. In reviewing our patient histories, we found that most of our cases diagnosed with fungal infection was related to agricultural work, therefore fungal keratitis was largely associated with farms [4,8,10,11]. China is a developing country in which farmers are still the largest part of the population overall. This fact may help explain why the incidence of fungal keratitis is higher in China. From our study and other previously published reports, it is clear that the prevalence of fungal keratitis is closely related to the developmental status of the economy and medical care. In less-developed countries, the incidence of fungal keratitis is beyond 30%, [8,11-13] whereas in developed countries, the corresponding incidence of fungal keratitis is between 6% and 20% of all microbial keratitis cases [8,14]. The major risk factor for infectious keratitis in the United States and the United Kingdom is contact lens application [1,8,14].

Fungal organisms can penetrate through the corneal stroma without perforating the cornea, resulting in an infectious hypopyon or endothelial plaque. In our study, the histopathlogic examination showed that hyphae distributed into corneal tissue extensively, including the superficial layer and stroma. Some of the hyphae invaded the full-thickness of the lamina, even breaking through Descemet's membrane. Furthermore, fungal infection accompanied with serious inflammatory cell infiltration. Therefore, corneal transplantation was performed in the majority of cases collected in the study.

Bacterial infection was the second most common corneal infection in current study; 279 cases (11.13%) with bacterial keratitis were found which is higher than an epidemiological study in Canada (all of infectious keratitis 5.6%) [5]. But it is lower than the reports from Bharathi MJ (by epithelial cell scraping) [15] and Vemuganti GK (by histology) [16] found that 34.4% and 23%, respectively, of corneal specimens with infectious keratitis were bacterial infection. The difference of incidence of bacterial keratitis may due to vary of the time of the corneal specimen collected, the geography and population. The lower incidence of bacterial infection in China may be due to improved living conditions, patient education, disease prevention, and the easy access to medical care, especially access to ophthalmologists for prompt treatment of infected corneas.

In the current study, the third most common histopathologic diagnosis of corneal infection was herpetic keratitis, ocular herpetic scar or recurrence is the most common reason for corneal transplantation especially in cases of recurrent interstitial herpetic keratitis. Previous studies reported that herpetic keratitis was considered to be the most common corneal disease leading to corneal transplantation in the 1990s [17,18]. In developed countries, the incidence and prevalence of herpetic ocular surface disease has been estimated to be approximately 2% of primary ocular HSV-1 infections [19] which is lower than what we found 10.30 % in current study. The lower incidence of herpetic keratitis may be due to the improvement of living condition and clinical treatment of herpetic keratitis [20-22]. Herpes virus can cause inflammation in all three layers of the cornea; however, stromal involvement is generally considered the most serious because of the risk of irreversible stromal scarring and subsequent visual loss. In reviewing the herptic keratitis cases we found there were lymphocytes infiltrating the stroma that is consistence with previous reports [9,20-22].

Acanthamoeba keratitis was the fourth most common infectious keratitis in our series, accounting for 1.04% (26 eyes). Acanthamoeba protozoa cysts and trophozoites were demonstrated using PAS staining. Bharathi et al [15] reported on 3183 eyes with infectious keratitis, 33 (1.04%) of which were diagnosed with acanthamoeba keratitis [15]. The incidence of acanthamoeba keratitis in the present investigation was similar to Bharathi's report, [15] although a corneal scarp was used for the detection of infectious pathogens in their study.

In our retrospective study, we found 567 eyes (22.63%) with agnogenic infectious keratitis. Godeiro et al [5] also reported that they were unable to determine the pathogenic organism in 45.6% of their keratitis cases by histopathologic examination of the infected corneas. There are a number of possible explanations as to why the cause of corneal infection could not be defined by the histopathologic methods. First, a fungal or bacterial infection couldn't be completely

excluded even with a negative PAS or Geimesa staining because the hyphae or bacteria may be significantly decreased or absent after long-term application of anti-fungal or anti-bacterial treatment. Second, there was possibility of a false negative result. And third, the rate of missed diagnosis could occur in clinical research.

In our study, corneal tumor (8.85%) was the second most common corneal disease diagnosed by histopathology; among the tumors highest incidence (58.81%) of subtype of corneal tumor was dermoid. This incidence of corneal dermoid was much higher than that (29%) incidence reported by Sunderraj [23]. Again our data suggest that the incidence of corneal disease is variable according population and geographic difference.

The most common histopathologic finding in other corneal disease was corneal leucoma (after wound or infections) accounting 23.18% over 768 eye among other corneal disease, rest of top six other corneal diseases such as marginal keratitis, Fuch's corneal dystrophy, Corneal degeneration, Chemical burn, Corneal edema and Bullous keratopathy after keratoplasty, Mooren's ulcer and included in our study were treated with surgical interventions such as corneal transplantation.

Of interesting, the histopathologic finding in our study was that only keratoconous (59 eyes, 3.18%) was demonstrated. A large investigation reported by Siganos, [6] showed that of the 2233 eyes included in their cases, 580 eyes (26%) were diagnosed with keratoconus. In a ten year period of study in Canada, Claire A. Sheldon show one of top five corneal diseases treated by corneal transplantation is keratoconous [24]. The variability in the incidence of keratoconus may be due to the difference in populations or ages involved in the study [25].

The classification of these 3592 corneal specimens from the Henan Eye Institute by pathological diagnosis could represent the prevalence of keratopathy in the central area of China since the patients involved in the study were from Henan Province and other surrounding provinces.

Taken together, our results showed that corneal infection, especially fungal keratitis, is the leading cause of infectious corneal disease and the major reason for corneal surgery. Our data can have important input in the field of corneal disease research in the Chinese population and, particularly, could aid in the development of targeted, individualized therapies and in the planning of treatment protocols and strategies.

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